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Too smart to fail – how viruses exploit the complexity of host cells during entry

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Biography Urs F. Greber, PhD

Urs Greber is a Professor for Cell Biology at the Institute of Life Sciences, Division of Biology of the University of Zürich, Switzerland. His research focuses on host factors and cellular trafficking supporting the infectious entry processes of non-enveloped viruses into cells, particularly human adenoviruses and picornaviruses.

Biography Francois-Loic Cosset, PhD

François-Loïc Cosset is a virologist from the National Center of Scientific Research (CNRS) at the Human Virology Department (Inserm U758) of the ENS of Lyon, France. His recent work concentrates on the assembly and entry of enveloped viruses, notably retroviruses and hepatitis C virus, and on the engineering of gene delivery vectors derived from pseudotyped lentiviruses.

Viruses are multivalent particles built of repetitively arranged proteins and sometimes sugars and lipids. They have various shapes and sizes ranging from dozens of nano-meters to micro-meters. Small viruses contain a minimal set of genes encoding structural and regulatory proteins and sometimes micro-RNAs, while large viruses have in addition complex genomes of hundreds of thousands of nucleotides.

To start their replication viruses go through sequential cell entry programs. This invariably involves attachment to one or several receptors and/or entry factors. Viral receptors are generally defined as cell surface molecules that can specifically bind components of the viral particles and mediate particle entry into cells, that is, into the cytosol. This definition for receptors has become unsuitable in many cases, for example owing to relatively low affinity and specificity interactions between certain “receptors” and viral components. For example, cell-associated lectins bind viral surface-expressed carbohydrates with low affinity, or low-density lipoprotein (LDL) receptors and scavenger receptor (SR)-BI bind cellular lipoprotein components associated to HCV particles. In addition, certain cell surface molecules are essential for virus entry, although they do not mediate virus binding *per se* [1]. On the other hand, the binding activity of some *bona fide* receptors has turned out to be nonessential for virus entry into cells [2]. This indicates that the events governing virus host interactions at the plasma membrane are more complex than initially anticipated. One level of recently appreciated complexity is the observation that productive receptor engagement often leads to patching of receptors by virus particles and triggers movements of extracellular viruses on the cell surface. Virus movements on the surface can facilitate the transmission of infectious particles between different cell types, or position virions to sites that are particularly proficient for signaling or internalization [3, 4]. Entry of the virus particles then typically proceeds in a stepwise fashion and culminates in the uncoating of the genome from the particle and infection.

Efficient entry is key for viral propagation. It is, however, antagonized by anti-viral defense mechanisms, which decode structural features of viral particles and trigger an

anti-viral response in the host cell even before viruses have entered cells. An important part of these innate anti-viral immune mechanisms are the so-called 'pattern recognition receptors' (PRRs). PRRs recognize pathogen associated molecular patterns (PAMPs). PRRs can have variable effects ranging from physical inactivation of pathogens to triggering complex intracellular signaling cascades, which lead to an anti-viral state of the host cell. Extracellular PRRs include a wide range of soluble proteins, such as pathogen-inactivating lectins [5], or defensins disrupting membranes or binding to capsid proteins [6]. Cell membrane associated PRRs comprise toll-like receptors, which recognize lipids or proteins on viral particles, and lectins, which bind to specific sugar moieties on viral glycoproteins or glycolipids.

How viruses take advantage of innate immunity to enter cells is discussed by Mathias Faure and Chantal Rabourdin-Combe. The authors elaborate on two main strategies, which bypass innate immunity mechanisms. One is evolutionary dynamics of viral structural proteins to evade recognition by PRRs. The other strategy is to usurp cell surface or endocytic PRRs as entry receptors, such as the mannose receptor or dendritic cell-specific ICAM grabbing non-integrin (DC-SIGN). Viral utilization of innate immunity receptors is of increasing interest, and illustrates the great versatility of viruses in the early stages of the life cycle.

The entry of enveloped viruses depends on viral proteins for attachment to cells, and fusion of the viral membrane with cellular membranes by specialized fusion proteins. Kouki Morizono and Irvin Chen discuss how particular receptors mediate two general mechanisms of pH-dependent and independent entry, exemplified by influenza virus and HIV, respectively. It has been possible to redirect the tropisms of the parent viruses and engineering viral surface glycoproteins used for pH-dependent or independent pathways, as illustrated in this review for the Sindbis virus E2 and the measles virus H glycoprotein.

Yuko Shimizu, Takayuki Hishiki, Saneyuki Ujino, Kazuo Sugiyama, Kenji Funami and Kunitada Shimotohno explore the role of host derived lipopropteins associated with

hepatitis C virus (HCV) isolates in opening the gates for the virus to enter hepatocytes and cause liver specific morbidity. HCV provides a very interesting example for how host factors can drive both the assembly of virus particle particles in hepatocytes, and upon contact with uninfected cells play an important role in virus entry. It is of note that HCV particles associate to lipoprotein-derived lipids and proteins, for example, apolipoproteins B, E, CI. This is critical for assembly of infectious virus and entry through receptors that bind the cellular lipoprotein components rather than the HCV glycoproteins.

Viral entry programs have often been evolved for specific pairs of viruses and cell types. For example, respiratory viruses target epithelial cells, hemorrhagic fever-causing emerging viruses infect endothelial cells, liver-tropic viruses infect hepatocytes or systemic disease causing viruses infect lymphoid cells. Under certain conditions, viruses also infect a large variety of different cell types and organisms. A broad tropism is a prerequisite for zoonotic transmissions and gives rise to emerging viruses when the viruses adapt to selective pressures in the new host. The review by Denis Gerlier discusses how emerging zoonotic viruses use receptors and entry pathways in their new hosts. A key for extended host range of these viruses appears to be evolutionary conserved orthologs of the receptors.

Most viruses are taken up by receptor-mediated endocytosis, akin to constitutive nutrient uptake or growth factor stimulated uptake. Viruses that are not strongly pH-dependent for their entry can use early endocytic compartments for escape to the cytosol. Other viruses that are more pH-dependent take longer rides in endocytic vesicles. They are referred to as late penetrating viruses. Late penetrating viruses receive cues for their uncoating and penetration programs in late endosomes or lysosomes and in rare instances the endoplasmic reticulum. Pierre-Yves Lozach, Jatta Huotari & Ari Helenius give a conceptual view of how late penetrating viruses use the endocytic system of mammalian cells. It is clear that deep cell biological insights are crucial for understanding how late penetrating viruses take advantage of this degradative cellular pathway. The authors describe four major cell biological alterations that occur when early

endosomes convert to late endosomes, namely acidification of the endosomal lumen, formation of luminal vesicles, the switch of Rab GTPases, and microtubule-mediated transport between these organelles. An important take home message is that low luminal pH is required for the proper trafficking and maturation of both early and late endosomes, and in particular for infection by late penetrating viruses. This concept is reinforced by the notion that viruses that are *per se* not dependent on low endosomal pH for infection can still be inhibited by perturbation of low pH in certain cell types.

Similar to fusion proteins from enveloped viruses, non-enveloped viruses encode membrane active proteins, which are incorporated into their capsids. These viruses undergo a stepwise uncoating program, which is controlled by cellular cues at particular locations, such as the plasma membrane, early endosomes or late endosomes. The membrane interacting proteins are exposed by receptor binding, acid, proteases or low calcium inside endosomes. For viruses that proceed into the ER, chaperones and redox conditions provide additional cues and change interchain disulfides in the viral capsid [7]. Further assistance by yet to be identified host factors is also required to release the viral genomes or the genome-containing capsids into the cytosol.

Crystal Moyer and Glen Nemerow discuss how a range of nonenveloped viruses uses distinct cues for their uncoating and membrane penetration. They discuss the single-stranded, positive-sense RNA viruses flock house virus, polio, coxsackie and rhinoviruses, the double-stranded RNA virus reovirus, and the DNA-viruses parvovirus, polyomavirus and adenovirus. Studies with these diverse viruses have uncovered three distinct mechanisms, by which these proteins interact with cellular membranes. One virus class functions by exposing an amphipathic alpha helix, for example the adenovirus protein VI, another one has a lipid anchor in the form of a myristoyl group, for example the poliovirus VP4 protein, and the third one appears to employ a catalytic function from a phospholipase, such as the parvoviral VP1 protein. Remarkably, all these strategies are broad enough to allow the viruses to infect many different cell types.

Conclusions

Proteins that build virus particles often work as ensembles rather than single molecules. A major challenge for future research is to elucidate how viral proteins cooperate during receptor mediated entry and membrane penetration. Any mechanistic understanding of the underlying cell biological processes will require functional integration of viral factors and cellular effectors in the context of live cells and eventually also in cell-free systems. The nature of some of the cellular factors is now emerging from various ‘omics technologies as well as genome-wide RNA interference screens [8, 9] [10, 11]. In many instances, these host factors control specific organelle properties, such as ion homeostasis, subcellular transport, formation and maintenance of membrane domains and membrane sorting processes [12]. Specific assays for distinct steps in virus entry and accurate assessments of viral entry intermediates coupled to subcellular analyses at high spatial resolution will have to be developed. This is helpful to determine, if particular host functions are directly or indirectly involved in virus entry and infection. The development of cell-free assays to study the role of host factors together with viral proteins will also contribute to clarifying the complexity of virus entry. Eventually, these approaches offer opportunities for new perturbations hopefully smarter than the viruses themselves.

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